

[0029] [Manufacturing Method for Substrate on which Nerve Cells are Arranged]

[0030] One embodiment of the present invention provides a manufacturing method for a substrate on which nerve cells are arranged, including a step of forming one or a plurality of liquid pools by arranging a plurality of liquid droplets containing nerve cells on a substrate using an inkjet method, to form one or a plurality of liquid pools, where the substrate has a region in which a cell adhesive material is arranged and a region in which a cell non-adhesive material is arranged; and a step of incubating the liquid pool until the nerve cells sediment and temporarily adhere onto the substrate to form a cell aggregate, where the diameter per one liquid pool is 500 μm or less and the density of nerve cells per one liquid pool is 10^5 cells/ cm^2 or more.

[0031] As will be described below in Examples, the inventors revealed that in a case where the diameter per one liquid pool which is arranged on the substrate is 500 μm or less and the density of nerve cells in the liquid pool is 10^5 cells/ cm^2 or more, the migration of nerve cells is suppressed and the nerve cells can be precisely arranged on the substrate. Further, in a case where a liquid droplet containing cells is ejected onto a substrate by an inkjet method, the cells can be stably arranged in the unit of several cells in a fine region in the order of magnitude of micrometers.

[0032] The diameter per one liquid pool may be 400 μm or less, 300 μm or less, or 200 μm or less. It is preferable to reduce the diameter of the liquid pool since it is easy to reduce the number of expensive cells to be used.

[0033] Here, the liquid droplet means a liquid droplet ejected from an inkjet head by the inkjet method. In addition, the liquid pool means a liquid droplet formed by landing a plurality of liquid droplets ejected from the inkjet head on the substrate. Further, the diameter per one liquid pool refers to the diameter of a region in which one liquid pool is in contact with the substrate. In a case where the region in which the liquid pool is in contact with the substrate is not circular, a circle having the same area as the region in which the liquid pools are in contact with the substrate is assumed, and the diameter thereof is used. The density of nerve cells per one liquid pool refers to the number of cells per area in which one liquid pool is in contact with the substrate.

[0034] The above liquid droplets preferably contain 1 to 50 nerve cells per one liquid droplet. In addition, the number of nerve cells contained in one liquid pool is preferably about 7 to 10,000. The number of nerve cells contained in one liquid pool may be 7 or more, 30 or more, or 70 or more. In addition, the number of nerve cells contained in one liquid pool may be 100 or less, 70 or less, or 30 or less. The upper limit and the lower limit thereof can be combined randomly.

[0035] The substrate is not particularly limited as long as it can be used for cell culture, and examples of the substrate material include organic materials and inorganic materials described below. These may be used alone or in a combination of two or more thereof.

[0036] The organic material is not particularly limited and may be appropriately selected depending on the intended purpose. Examples thereof include polyethylene terephthalate (PET), polystyrene (PS), polycarbonate (PC), triacetyl cellulose (TAC), polyimide (PI), nylon (Ny), low-density polyethylene (LDPE), medium-density polyethylene (MDPE), vinyl chloride, vinylidene chloride, polyphenylene sulfide, polyether sulfone, polyethylene naphthalate, polypropylene, an acrylic material such as urethane acrylate,

cellulose, a silicone-based material such as polydimethylsiloxane (PDMS), polyvinyl alcohol (PVA), a metal alginate salt such as calcium alginate, and gel-like materials such as polyacrylamide, methyl cellulose, and agarose.

[0037] The inorganic material is not particularly limited and may be appropriately selected depending on the intended purpose. Examples thereof include glass and ceramics.

[0038] The structure of the substrate is not particularly limited as long as it can be used for cell culture, and examples thereof include a porous structure and a non-porous structure. The substrate may be a substrate having a porous structure or may be a substrate having a non-porous flat plate member in which a porous member is laminated.

[0039] The size and the shape of the fine pores of the porous structure are not limited and, for example, a mesh structure, an uneven structure, a honeycomb structure, or the like may be adopted. The substrate structure is preferably a porous structure since the surface area on which a cell non-adhesive material or a cell adhesive material is fixed is large, a large amount of solution can be retained, and drying can be suppressed.

[0040] In a case where a substrate having a porous structure is used as the substrate and a liquid pool is formed in a state where a liquid such as a medium is retained in the substrate in advance, the liquid is retained by the above-described porous structure, and thus drying of the liquid pool can be suppressed.

[0041] Further, in a case where liquid droplets are ejected onto the dried substrate to form a liquid pool, the shape of the liquid pool can be maintained and the adhesion of the cells on the substrate can be stably achieved by carrying out a step of suppressing the evaporation of the liquid in the liquid pool (drying-suppressing step).

[0042] Examples of the drying-suppressing step include (i) a step of increasing the humidity in the vicinity of the liquid pool, (ii) a step of forming the liquid pool after arranging a fluid that suppresses evaporation of the liquid on the substrate (here, examples of the fluid that suppresses evaporation of the liquid include oil, medium, and a buffer solution), (iii) a step of coating the liquid pool with a fluid (for example, oil) that suppresses evaporation of the liquid after forming the liquid pool on the substrate.

[0043] In a case where the suppression of evaporation is carried out by increasing the humidity, it is preferable to perform local humidity control in order to minimize the influence on the surroundings. In addition, in a case where oil is used to suppress drying, it is preferable to use oil that has biocompatibility in terms of suppressing the influence on cells.

[0044] The manufacturing method of the present embodiment may further include a step of supplying a medium to the substrate on which the cell aggregate is formed. As the medium, a medium suitable for the cells to be used can be appropriately selected and used. Specific examples of the medium include Dulbecco's modified Eagle's medium (DMEM), Ham's F-12 medium (Ham's Nutrient Mixture F12), D-MEM/F12 medium, McCoy's 5A medium, Eagle's minimum essential medium (EMEM), alpha modified Eagle's minimum essential medium (aMEM), Minimum essential medium (MEM), Roswell Park Memorial Institute-1640 medium (RPMI 1640), Iscove's modified Dulbecco's medium (IMDM), MCDB 131 medium, William's medium E, IPL41 medium, Fischer's medium, M199 medium, High